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REVISION OF THE ABSOLUTE CONFIGURATION OF A-FACTOR

THE INDUCER OF STREPTOMYCIN BIOSYNTHESIS, BASING ON THE RECONFIRMED (*R*)-CONFIGURATION OF (+)-PARACONIC ACID†

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Abstract—(+)-Paraconic acid was shown to be of (*R*)-configuration by its four-step conversion to (*R*)-(+)-3-methyl-4-butanolide. The absolute configuration at C-3 of A-factor [2-(6-methylheptanoyl)-3-hydroxymethyl-4-butanolide] was therefore revised to be *R*, since it was synthesized from (*S*)-(−)-paraconic acid.

Recently, we reported a synthesis of the optically active forms of A-factor **1**.[1] This lactone was first isolated by Khokhlov *et al.* as the inducer of the biosynthesis of streptomycin in inactive mutants of *Streptomyces griseus*.[2] The role of A-factor in streptomycin production was further studied by Hara and Beppu.[3,4] The gross structure of A-factor **1** was determined on the bases of the structural studies[5] and a synthesis of (±)-**1**[6] by the Russian workers. They also proposed the absolute configuration of A-factor to be 3*S*[5,7] applying the Klyne's lactone sector rule.[8] Since A-factor can exist either as a keto-form **1A** or as an enol-form **1B**, the applicability of the lactone sector rule is not unambiguous.

Our synthesis of (−)-A-factor **1a** from (−)-paraconic acid **2** established the identity of (−)-**1** with the natural A-factor.[1] This means that the absolute configuration at C-3 of A-factor is same as that of (−)-paraconic acid. The optical resolution of (±)-paraconic acid was fully described by Tocanne and Asselineau in 1965 as cited in two well-known monographs on optical resolution.[10,11] The French workers converted (−)-paraconic acid **2** to (*S*)-(−)-2-methylsuccinic acid **3** in two steps, establishing the absolute configuration of (−)-**2** to be *R*.[9] Basing on their result we assigned (3*S*)-configuration to (−)-A-factor **1**.[1]

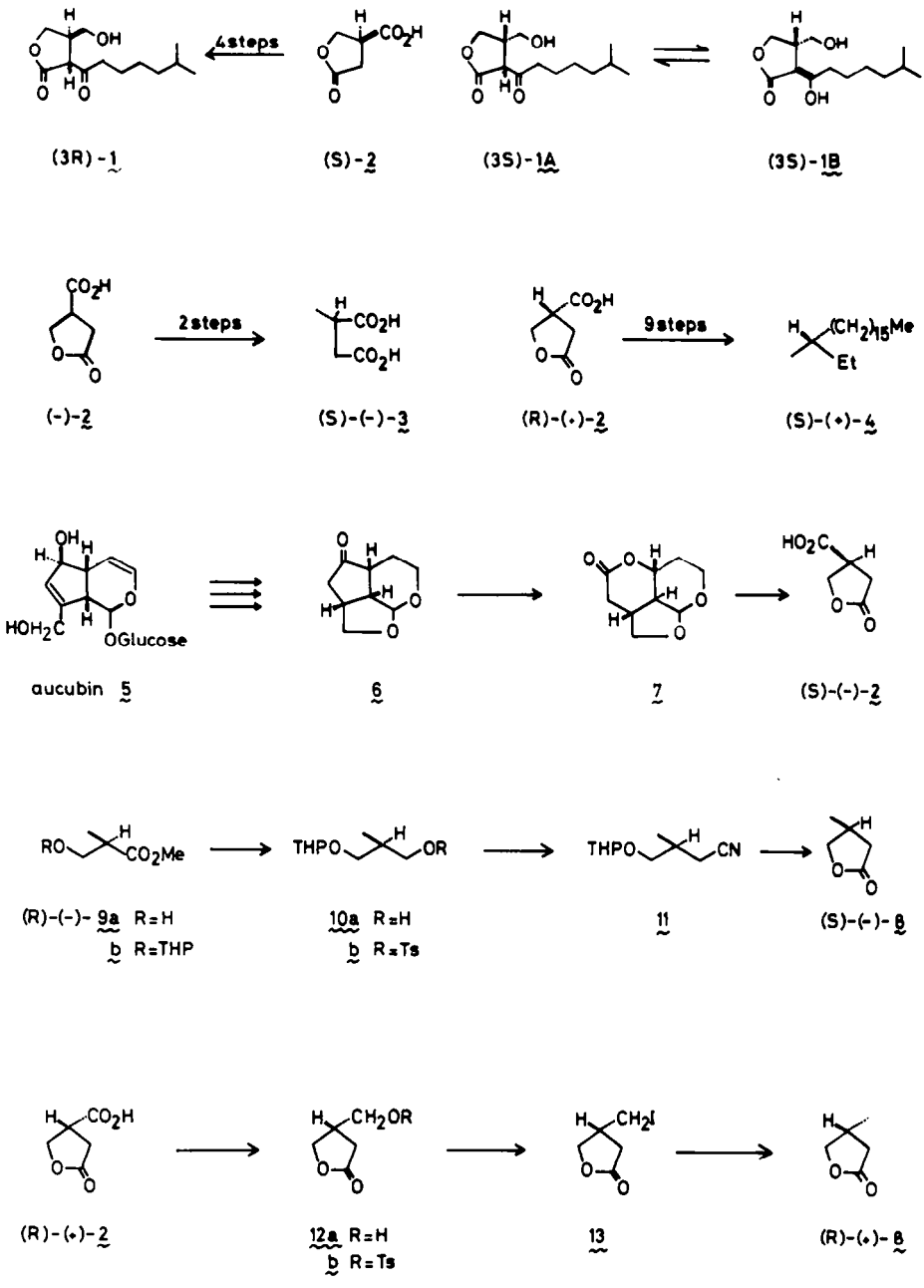
There are some other papers, however, where (*R*)-configuration was assigned to (+)-paraconic acid **2**.[12,13]‡ This assignment was based on the formation of (−)-paraconic acid **2** from aucubin **5**.[14,15] Tetrahydroanhydroaucubigenone **6** derived from **5** was submitted to the Baeyer-Villiger oxidation to give **7**, whose $KMnO_4$ oxidation yielded (−)-**2**.[14] Since the absolute configuration of **6** was deduced to be as depicted in the formula by comparing its ORD data with those of steroidal and sesquiterpenoidal cyclopentanones of known absolute stereochemistry, (*S*)-configuration was given to (−)-paraconic acid **2**.[15] This assignment was employed for the stereo-chemical studies on pterocarpinoids[12] and isoflavonoids[13] as cited by Klyne and Buckingham.[16]

The above described controversial situation concerning the stereochemistry of paraconic acid was apparently settled by the later result of Tocanne and Asselineau correlating (+)-paraconic acid **2** to (*S*)-(+)-3-methylnonadecane **4** by a complicated nine-step process including a malonic ester synthesis and a Kolbe electrolysis. They therefore concluded that (+)-paraconic acid **2** possesses (*R*)-configuration[17] contrary to their earlier result.[9]

In view of the key role of paraconic acid **2** in assigning the absolute configuration of various natural products including A-factor, it seemed desirable to establish a more direct and unambiguous correlation of **2** with a compound of known absolute stereochemistry. We therefore planned the conversion of paraconic acid **2** into 3-methyl-4-butanolide **8**. Although the latter with known absolute configuration was available either by microbial transformation[18] or by asymmetric synthesis,[19] a new synthesis was devised so as to secure a reference sample for direct comparison. Our starting material was methyl (*R*)-(−)-3-hydroxy-2-methylpropionate **9a**, which was readily available by methylating the microbial oxidation product of isobutyric acid with *Candida rugosa* IFO 0750.[20] The ester **9a** (97% e.e. as determined by HPLC analysis of its (*S*)-α-methoxy-α-trifluoromethylphenylacetate[21]) was converted to a THP ether **9b**. This was reduced with LAH to **10a**. The corresponding tosylate **10b** was treated with NaCN to give a nitrile **11**. Alkaline hydrolysis of **11** was followed by acidification to afford (*S*)-(−)-**8**, $[\alpha]_D^{21}$ − 24.96° (MeOH) [lit.[18] $[\alpha]_D^{20}$ − 24.7° (MeOH)]. Having this reference sample in hand, the next task was the conversion of paraconic acid **2** to the lactone **8**. (+)-Paraconic acid **2**, $[\alpha]_D^{21}$ + 61.55° (MeOH), was reduced with $BH_3 \cdot Me_2S$ to (+)-hydroxy lactone **12a** as described previously.[1] The corresponding tosylate **12b** was treated with LiI to give an iodolactone **13**. This was hydrogenolyzed over Raney Ni W-7[22] to yield (*R*)-(+)-**8**, $[\alpha]_D^{21.5}$ + 22.97° (MeOH) [lit.[19] $[\alpha]_D^{25}$ + 23° (MeOH)]. Its IR and NMR spectra were identical to those of the reference sample. The identity was also proved by GLC analysis. This showed beyond doubt that (+)-paraconic acid belongs to the (*R*)-series. The present result is contrary to the conclusion of the original French work[9], but consistent with both the later French work[17] and the Japanese work.[12,14,15] In the earlier French work[9] there

†Synthetic microbial chemistry—III. Part II, K. Mori and K. Yamane, *Tetrahedron* **38**, 2919 (1982).

‡Dr. J. Buckingham, the co-author of *Atlas of Stereochemistry*, kindly brought these papers to my attention in his personal communication dated 20 January 1983.



must have been some anomaly as the authors themselves admitted later.[17]

In conclusion, our previous result on the absolute configuration at C-3 of A-factor **1** must now be revised. Since the natural (−)-A-factor **1** was synthesized from (−)-paraconic acid with (*S*)-configuration, the absolute configuration at C-3 of (−)-**1** is *R*. Therefore the natural A-factor should be called (3*R*)-(−)-A-factor **1**. This conclusion is contrary to Khokhlov's proposal basing on the lactone sector rule.[5,7]

EXPERIMENTAL

All b.ps were uncorrected. IR spectra were recorded on a Jasco A-102 spectrometer as film. NMR spectra were recorded on a Hitachi R-24A spectrometer at 60 MHz with TMS as an internal standard. Optical rotations were measured on a Jasco DIP-140 automatic polarimeter,

Methyl (R)-(−)-3-*hydroxy*-2-*methylpropionate THP ether* **9b**. The starting material (*R*)-(−)-**9a**, $[\alpha]_D^{25}$ − 26.3° (*c* = 2, MeOH), was a gift from Kanegafuchi Chemical Industry Co., Ltd., Takasago, Hyogo 676. Its optical purity was determined by the HPLC analysis of the corresponding (*S*)-MTPA ester:[21] Apparatus, Shimadzu LC-2; Column, Partisil 5, 25 cm × 4.6 mm; Eluent: n-hexane-THF-MeOH (6000:100:1), 15 kg/cm^2; detection at 217 nm: R_t 74.8 min (1.5%), 82.4 min (98.5%). Optical purity = 97%. *p*-TsOH (0.1 g) was added to a soln of **9a** (5.9 g) and dihydropyran (5.0 g) in ether (50 ml) and the mixture was left to stand overnight at room temp. The soln was washed with $NaHCO_3$ aq, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 10.0 g (99%) of **9b**, b.p. 112 ~ 115°/12 mm, n_D^{21} 1.4391; $[\alpha]_D^{21}$ − 16.3° (*c* = 1.39, ether); ν_{max} 1745 (vs), 1200 (s), 1130 (s), 1125 (s), 1080 (s), 1060 (s), 1035 (vs) cm^{-1}; δ (CCl_4) 1.12 (3H, d, *J* = 7 Hz), 1.25 ~ 1.90 (6H, br.), ~ 2.64 (1H, m), 3.62 (3H, s), 3.12 ~ 3.96 (4H, m), 4.52 (1H, br. s). (Found: C, 59.63; H, 9.03. Calc for $C_{10}H_{18}O_4$: C, 59.38; H, 8.97%).

(S)-(−)-2-*Methyl*-1,3-*propanediol mono THP ether* **10a**. A soln of **9b** (10.0 g) in dry ether (50 ml) was added dropwise to a stirred suspension of LAH (1.5 g) in dry ether (100 ml) at 0 ~ 5°. The stirring was continued for 4 hr at room temp. The excess LAH was decomposed by the successive addition of water (1.5 ml), 15% NaOHaq (1.5 ml) and water (4.5 ml) to the stirred and ice-cooled mixture. After stirring for 1.5 hr, the mixture was filtered and the solid was washed with ether. The combined filtrate and washings were dried (K_2CO_3) and concentrated *in vacuo*. The residue was distilled to give 8.2 g (95%) of **10a**, b.p. 105 ~ 107°/7 mm, n_D^{21} 1.4522; $[\alpha]_D^{21}$ − 1.2° (*c* = 1.471, ether); ν_{max} 3440 (m), 1140 (s), 1125 (s), 1080 (s), 1060 (s), 1035 (vs) cm^{-1}; δ (CCl_4) 0.90 (3H, *d*, J = 7 Hz), 1.3 ~ 2.2 (7H), ~ 2.6 (1H, br.), 3.1 ~ 4.1 (6H, m), 4.58 (1H, br. s). (Found: C, 62.04; H, 10.41. Calc for $C_9H_{18}O_3$: C, 62.04; H, 10.41%.)

(R)-2-*Methyl*-1,3-*propanediol mono THP ether tosylate* **10b**. *p*-TsCl (12 g) was added to a stirred soln of **10a** (8.2 g) in dry C_5H_5N (60 ml) at 0 ~ 5°. The mixture was left to stand overnight in a refrigerator, and then poured into iced-water and extracted with ether. The ether soln was washed with water, $CuSO_4$ soln, $NaHCO_3$ soln and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 16.0 g (97.6%) of **10b**, ν_{max} 1605 (m), 1365 (s), 1195 (vs), 1180 (vs), 1040 (s), 980 (s), 820 (s), 670 (s) cm^{-1}. This was employed in the next step without further purification.

(S)-(−)-4-*Hydroxy*-3-*methylbutanenitrile THP ether* **11**. NaCN (4.0 g) was added to a soln of **10b** (16.0 g) in DMSO (40 ml). The mixture was stirred overnight at room temp. It was then diluted with water and extracted with n-pentane. The extract was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was distilled to give 7.1 g (84.5%) of **11**, b.p. 112 ~ 115°/7 mm, $n_D^{21.5}$ 1.4477; $[\alpha]_D^{21.5}$ − 27.8° (*c* = 1.41, ether); ν_{max} 2240 (w), 1135 (m), 1125 (m), 1080 (m), 1065 (m), 1035 (s), 1020 (m) cm^{-1}; δ (CCl_4) 1.10 (3H, d, *J* = 7 Hz), 1.3 ~ 1.9 (6H, br.), 1.9 ~ 2.25 (1H, m), 2.25 ~ 2.55 (2H, m), 3.05 ~ 4.10 (4H, m), 4.60 (1H, br. s). (Found: C, 65.39; H, 9.39; N, 7.15. Calc for $C_{10}H_{17}O_2N$: C, 65.54; H, 9.35; N, 7.64%.)

(S)-(−)-3-*Methyl*-4-*butanolide* **8**. A soln of KOH (3 g) in water (3 ml) was added to a soln of **11** (7.1 g) in ethylene glycol (7 ml). The mixture was stirred and heated under reflux for 6 hr. After cooling, the soln was acidified with conc HCl (*ca* 6 ml) and heated at 100° for 10 min. It was then made alkaline with KOH (*ca* 3 g) and extracted with $CHCl_3$ to remove neutral impurities. The aq layer was acidified with dil HCl, saturated with NaCl and extracted with $CHCl_3$. The extract was washed with NaCl–$NaHCO_3$ soln, dried ($MgSO_4$) and concentrated *in vacuo* to give 2.5 g of a crude neutral oil. This was chromatographed over SiO_2 (Merck Kieselgel 60, 70 ~ 230 mesh, 35 g, 10 × 3.5 cm) in *n*-pentane. Elution with *n*-pentane-ether (2:1 ~ 1:1) gave **8**. Distillation gave 908 mg (23.4%) of pure (*S*)-(−)-**8**, b.p. 96 ~ 97°/28 mm, n_D^{21} 1.4295; $[\alpha]_D^{21}$ − 24.96° (*c* = 1.771, MeOH) [lit.[18] $[\alpha]_D^{20}$ − 24.7° (*c* = 4, MeOH)]; ν_{max} 1780 (s), 1170 (s), 1020 (s), 995 (s) cm^{-1}; δ (CCl_4) 1.15 (3H, *d*, J = 6 Hz), 1.7 ~ 2.95 (3H, m), 3.6 ~ 4.5 (2H, m); GLC (Column, PEG 20 M, 2m × 4 mm at 115°; Carrier gas, N_2, 1.4 kg/cm^2): R_t 3.7 min (100%). (Found: C, 59.41; H, 7.86. Calc for $C_5H_8O_2$: C, 59.98; H, 8.05%.)

(S)-(+)-3-*Hydroxymethyl*-4-*butanolide* **12a**. (+)-Paraconic acid, $[\alpha]_D^{21}$ + 61.55° (MeOH), was prepared by Mr. K. Yamane in January 1982.[1] By the reported procedure,[1] (+)-**2** (1.2571 g) gave 1.06 g of crude **12a** $[\alpha]_D^{21}$ + 37.5° (*c* = 1.551, $CHCl_3$), ν_{max} 3420 (m), 1770, (s), 1180 (s), 1030 (s) cm^{-1}. This was employed in the next step without further purification.

(R)-3-p-*Tosyloxymethyl*-4-*butanolide* **12b**. *p*-TsCl (3.1 g) was added to a stirred soln of **12a** (1.06 g) in dry C_5H_5N (12 ml) at 0 ~ 5°. The mixture was left to stand overnight in a refrigerator, and then diluted with iced-water and extracted with ether. The ether soln was washed with dil HCl, $NaHCO_3$aq and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 1.6 g (62%) of crude **12b**, ν_{max} 1780 (s), 1595 (m), 1355 (s), 1185 (s), 1170 (s) cm^{-1}. This was employed in the next step without further purification.

(R)-3-*Iodomethyl*-4-*butanolide* **13**. LiI (3.0 g) was added to a soln of **12b** (1.6 g) in acetone (20 ml). The soln was stirred overnight at room temp and then concentrated *in vacuo*. The residue was diluted with water and extracted with EtOAc. The extract was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo* to give 1.3 g (quantitative) of crude **13**, ν_{max} 1780 (s), 1225 (m), 1170 (s), 1020 (m) cm^{-1}. This was employed in the next step without further purification.

(R)-(+)-3-*Methyl*-4-*butanolide* **8**. Raney Ni W-7 (3.5 g) and powdered $CaCO_3$ (0.5 g) were added to a stirred soln of **13** (1.3 g) in 99% EtOH (20 ml). The mixture was stirred for 30 min at room temp and filtered. The catalyst was washed with 99% EtOH. The combined filtrate and washings were concentrated *in vacuo*. The residue was chromatographed over SiO_2 (Merck Kieselgel 60, 70 ~ 230 mesh, 7 g, 4 × 2.5 cm) in n-hexane. Elution with n-hexane–ether (1:1) gave 300 mg of **8**. This was distilled to give 50 mg (8.8%) of pure (*R*)-(+)-**8**, b.p. 120°/45 mm, n_D^{21} 1.4420; $[\alpha]_D^{21.5}$ + 22.97° (*c* = 0.37, MeOH) [lit.[19] $[\alpha]_D^{25}$ + 23° (*c* = 4.2, MeOH)]. This showed the IR and NMR spectra completely identical to those of (*S*)-(−)-**8**. The identity was also confirmed by GLC co-injection experiment on a PEG 20 M column.

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